Excerpt from full transcript including search strategy

FILE 'USPAT' ENTERED AT 12:55:14 ON 23 JUL 1999

6. 5,648,247, Jul. 15, 1997, Method for increasing the omega-hydroxylase
activity in candida tropicals; Stephen Picataggio, et al., 435/142,
254.22 [IMAGE AVAILABLE]
8. 5,620,878, Apr. 15, 1997, Method for increasing the omega-hydroxylase
activity in Candida tropicalis; Stephen Picataggio, et al., 435/142,
254.22 [IMAGE AVAILABLE]
L1 0 S PICHIA PASTORIS (P) CYTOCHROME P450 (P) (TRANSFORM? OR T
RAN
L2 0 S PICHIA PASTORIS (P) MONOOXYGENASE (P) (TRANSFORM? OR
TRA
NSF
L3 9 S (YEAST OR CANDIDA MALTOSA) (P) CYTOCHROME P450 (P)
(TRAN
SFO
L4 47145 S ALKANE HYDROXYLAT? OR DICARBOXYL?
L5 13 S L4 AND CYTOCHROME P450
L6 11 S L5 NOT L3
L7 3 S POX4 AND URA3
L8 70 S CANDIDA MALTOSA
L9 48 S L8 AND HOST CELL
L10 39 S L9 AND HETEROLOG?
L11 1 S CANDIDA MALTOSA /TI

U.S. Patent & Trademark Office LOGOFF AT 13:13:53 ON 23 JUL 1999

FILE 'HOME' ENTERED AT 13:13:32 ON 23 JUL 1999

=> file medline, biosis, caplus, agricola

L1 ANSWER 1 OF 2 MEDLINE
AN 96154241 MEDLINE
DN 96154241
TI Functional expression of recombinant spiny dogfish shark (Squalus
acanthias) cytochrome P450c17 (17 alpha-hydroxylase/C17,20-lyase)
in yeast
(Pichia pastoris).
AU Trant J M
CS Department of Zoology and Physiology, Louisiana State University,
Baton
Rouge 70803, USA.. trant@umbi.umd.edu
SO ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, (1996 Feb 1) 326 (1) 8-
14.
Journal code: 6SK. ISSN: 0003-9861.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199605
AB The cDNA encoding the spiny dogfish shark (Squalus acanthias)
testicular

form of cytochrome P450c17 (CYP17) was used to direct the heterologous expression of a functional enzyme in yeast (***Pichia*** ***pastoris***). This protein possesses two enzymatic activities: 17 alpha-hydroxylase and C17,20-lyase reactions. Cytochrome P450c17 is a key steroidogenic enzyme for the production of sex steroids in gonadal tissue and for cortisol production in adrenal tissue. This study describes the culture conditions and the enzymatic activity of ***recombinant*** shark cytochrome P450c17. The shark enzyme was compatible with the endogenous yeast NADPH- ***cytochrome*** ***P450*** reductase and was bioactive within the living yeast cell. Progesterone (at 15 microM) was metabolized (51 pmol/min/10(9) cells) faster than pregnenolone (36 pmol/min/10(9) cells). Both progesterone and pregnenolone were completely metabolized to their respective androgens (androstenedione and dehydroepiandrosterone). Although 11 beta-hydroxy-progesterone was readily 17 alpha-hydroxylated by the shark P450, the lyase reaction was not evident. Alterations to the 2-carbon sidechain of progesterone (21-hydroxylation or 20 beta-reduction) prevented metabolism. High-density cultures (> 1.5 x 10(9) cells/ml) yielded the greatest quantity of ***recombinant*** protein but cultures of lower density produced more ***recombinant*** protein per cell. This is the first report of heterologous expression in yeast of a steroidogenic ***cytochrome*** ***P450*** from a lower vertebrate.

L6 ANSWER 9 OF 13 CAPLUS COPYRIGHT 1999 ACS
AN 1988:524518 CAPLUS
DN 109:124518
TI Degradation of long-chain n-alkanes by the yeast ***Candida*** ***maltosa*** . II. Oxidation on n-alkanes and intermediates using microsomal membrane fractions
AU Blasig, R.; Mauersberger, S.; Riege, P.; Schunck, W. H.; Jockisch, W.; Franke, P.; Mueller, H. G.
CS Cent. Inst. Mol. Biol., Ger. Acad. Sci., Berlin, DDR-1115, Ger. Dem. Rep.
SO Appl. Microbiol. Biotechnol. (1988), 28(6), 589-97
CODEN: AMBIDG; ISSN: 0175-7598
DT Journal
LA English
AB Microsomal membrane fractions of the yeast C. maltosa were investigated

with respect to their ability to catalyze the oxidn. of n-alkanes, fatty alcs. and fatty acids. Anal. of intermediates of n-hexadecane oxidn. led to the conclusion that monoterminal attack was predominant, whereas diterminal oxidn. proceeded as a minor reaction. The oxidn. of long-chain primary alcs. to the corresponding aldehydes occurred without addn. of NAD (phosphate) [NAD(P)+] and was accompanied by stoichiometric oxygen consumption and hydrogen peroxide prodn., suggesting that an alc. oxidase instead of an NAD(P)+-requiring alc. dehydrogenase catalyzed these reactions. As shown for n-hexadecane, the hydroxylation of palmitic acid was found to be carbon monoxide-dependent, indicating involvement of a cytochrome P 450 system, as in the case of n- ***alkane*** ***hydroxylation*** .

L6 ANSWER 10 OF 13 CAPLUS COPYRIGHT 1999 ACS
AN 1987:210770 CAPLUS
DN 106:210770
TI Function and regulation of cytochrome P-450 in alkane-assimilating yeast.
II. Effect of oxygen-limitation
AU Schunck, W. H.; Mauersberger, S.; Kaergel, E.; Huth, J.; Mueller, H. G.
CS Cent. Inst. Mol. Biol., Ger. Acad. Sci., Berlin-Buch, DDR-1115, Ger. Dem. Rep.
SO Arch. Microbiol. (1987), 147(3), 245-8
CODEN: AMICCW; ISSN: 0302-8933
DT Journal
LA English
AB Transition of n-hexadecane utilizing cultures of ***Candida*** ***maltosa*** to oxygen-limited growth caused an .ltoreq.6-fold increase of the cellular cytochrome P 450 content. Enhanced cytochrome P 450 formation required protein de novo synthesis and was not due to a change of the apo/holo-enzyme ratio as demonstrated by cycloheximide inhibition and immunol. quantitation. The effect of low oxygen concn. (pO2 = 3-5%) was simulated by selective inhibition of ***alkane*** ***hydroxylation*** with carbon monoxide (at a pO2 of 70-75%). Enhanced cytochrome P 450 formation occurred even when a const. growth rate was maintained through utilization of a second nonrepressive growth substrate. However, the presence of n-alkanes was an essential precondition. Apparently, the cytochrome P 450 formation was mainly regulated by the

intracellular inducer concn. which depends on the relative rates of alkane
transport into the cell and the actual ***alkane***
hydroxylating activity of the enzyme system.

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FILE 'MEDLINE, BIOSIS, CAPLUS, AGRICOLA' ENTERED AT 13:14:54 ON 23 JUL 1999

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L1             2 S PICHIA PASTORIS (P) CYTOCHROME P450 (P)(TRANSFORM? OR
TANSFEC
L2             0 S PICHIA PASTORIS (P) MONOOXYGENASE (P) (TRANSFORM? OR
TRANSFEC
L3         55430 S ALKANE HYDROXYLAT? OR DICARBOXY?
L4            75 S L3 AND CYTOCHROME P450
L5            13 S L4 AND CANDIDA MALTOSA
L6            13 S L5 NOT L1
L7             5 S POX4 AND URA3
L8             5 S L7 NOT L1
L9             5 S L7 NOT L6
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=> log y

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STN INTERNATIONAL LOGOFF AT 13:19:42 ON 23 JUL 1999